<110> Hildinger, Markus

<120> Decreasing gene expression in a mammalian subject in vivo via
       AAV-mediated RNAi expression cassette transfer

<130> 2

<140> US 10/604,340
<141> 2003-07-13

<160> 12

<170> PatentIn version 3.2

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       expression cassette; referred to as AAV2/2 CMV luciferase

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Arg Phe His Leu Pro Gly Ile Arg Gln Gly Tyr Gly Leu Thr Glu Thr
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Val Asp Ile Thr Tyr Ala Glu Tyr Phe Glu Met Ser Val Arg Leu Ala
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Glu Ala Met Lys Arg Tyr Gly Leu Asn Thr Asn His Arg Ile Val Val
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Cys Ser Glu Asn Ser Leu Gln Phe Phe Met Pro Val Leu Gly Ala Leu
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Phe Ile Gly Val Ala Val Ala Pro Ala Asn Asp Ile Tyr Asn Glu Arg
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Glu Leu Leu Asn Ser Met Gly Ile Ser Gln Pro Thr Val Val Phe Val
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Phe Thr Asp Ala His Ile Glu Val Asp Ile Thr Tyr Ala Glu Tyr Phe
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Phe Asp Arg Asp Lys Thr Ile Ala Leu Ile Met Asn Ser Ser Gly Ser
            190             195             200

act ggt ctg cct aaa ggt gtc gct ctg cct cat aga act gcc tgc gtg    1878
Thr Gly Leu Pro Lys Gly Val Ala Leu Pro His Arg Thr Ala Cys Val
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aga ttc tcg cat gcc aga gat cct att ttt ggc aat caa atc att ccg    1926
Arg Phe Ser His Ala Arg Asp Pro Ile Phe Gly Asn Gln Ile Ile Pro
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gat act gcg att tta agt gtt gtt cca ttc cat cac ggt ttt gga atg    1974
Asp Thr Ala Ile Leu Ser Val Val Pro Phe His His Gly Phe Gly Met
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ttt act aca ctc gga tat ttg ata tgt gga ttt cga gtc gtc tta atg    2022
Phe Thr Thr Leu Gly Tyr Leu Ile Cys Gly Phe Arg Val Val Leu Met
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tat aga ttt gaa gaa gag ctg ttt ctg agg agc ctt cag gat tac aag    2070
Tyr Arg Phe Glu Glu Glu Leu Phe Leu Arg Ser Leu Gln Asp Tyr Lys
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att caa agt gcg ctg ctg gtg cca acc cta ttc tcc ttc ttc gcc aaa    2118
Ile Gln Ser Ala Leu Leu Val Pro Thr Leu Phe Ser Phe Phe Ala Lys
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agc act ctg att gac aaa tac gat tta tct aat tta cac gaa att gct    2166
Ser Thr Leu Ile Asp Lys Tyr Asp Leu Ser Asn Leu His Glu Ile Ala
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tct ggt ggc gct ccc ctc tct aag gaa gtc ggg gaa gcg gtt gcc aag    2214
Ser Gly Gly Ala Pro Leu Ser Lys Glu Val Gly Glu Ala Val Ala Lys
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agg ttc cat ctg cca ggt atc agg caa gga tat ggg ctc act gag act    2262
Arg Phe His Leu Pro Gly Ile Arg Gln Gly Tyr Gly Leu Thr Glu Thr
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Val Gly Lys Val Val Pro Phe Phe Glu Ala Lys Val Val Asp Leu Asp
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acc ggg aaa acg ctg ggc gtt aat caa aga ggc gaa ctg tgt gtg aga        2406
Thr Gly Lys Thr Leu Gly Val Asn Gln Arg Gly Glu Leu Cys Val Arg
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Gly Pro Met Ile Met Ser Gly Tyr Val Asn Asn Pro Glu Ala Thr Asn
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gcc ttg att gac aag gat gga tgg cta cat tct gga gac ata gct tac        2502
Ala Leu Ile Asp Lys Asp Gly Trp Leu His Ser Gly Asp Ile Ala Tyr
410             415             420             425

tgg gac gaa gac gaa cac ttc ttc atc gtt gac cgc ctg aag tct ctg        2550
Trp Asp Glu Asp Glu His Phe Phe Ile Val Asp Arg Leu Lys Ser Leu
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Leu Leu Gln His Pro Asn Ile Phe Asp Ala Gly Val Ala Gly Leu Pro
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Asp Asp Asp Ala Gly Glu Leu Pro Ala Ala Val Val Val Leu Glu His
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Gly Lys Thr Met Thr Glu Lys Glu Ile Val Asp Tyr Val Ala Ser Gln
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Val Thr Thr Ala Lys Lys Leu Arg Gly Gly Val Val Phe Val Asp Glu
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Val Pro Lys Gly Leu Thr Gly Lys Leu Asp Ala Arg Lys Ile Arg Glu
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Ile Leu Ile Lys Ala Lys Lys Gly Gly Lys Ile Ala Val
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Val Asp Ile Thr Tyr Ala Glu Tyr Phe Glu Met Ser Val Arg Leu Ala
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Glu Ala Met Lys Arg Tyr Gly Leu Asn Thr Asn His Arg Ile Val Val
65              70              75              80

Cys Ser Glu Asn Ser Leu Gln Phe Phe Met Pro Val Leu Gly Ala Leu
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Phe Ile Gly Val Ala Val Ala Pro Ala Asn Asp Ile Tyr Asn Glu Arg
            100             105             110

Glu Leu Leu Asn Ser Met Gly Ile Ser Gln Pro Thr Val Val Phe Val
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Ser Lys Lys Gly Leu Gln Lys Ile Leu Asn Val Gln Lys Lys Leu Pro
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Ile Ile Gln Lys Ile Ile Ile Met Asp Ser Lys Thr Asp Tyr Gln Gly
145             150             155             160

Phe Gln Ser Met Tyr Thr Phe Val Thr Ser His Leu Pro Pro Gly Phe
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Asn Glu Tyr Asp Phe Val Pro Glu Ser Phe Asp Arg Asp Lys Thr Ile
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Ala Leu Ile Met Asn Ser Ser Gly Ser Thr Gly Leu Pro Lys Gly Val
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Ala Leu Pro His Arg Thr Ala Cys Val Arg Phe Ser His Ala Arg Asp
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Pro Ile Phe Gly Asn Gln Ile Ile Pro Asp Thr Ala Ile Leu Ser Val
225             230             235             240

Val Pro Phe His His Gly Phe Gly Met Phe Thr Thr Leu Gly Tyr Leu
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Ile Cys Gly Phe Arg Val Val Leu Met Tyr Arg Phe Glu Glu Glu Leu
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Phe Leu Arg Ser Leu Gln Asp Tyr Lys Ile Gln Ser Ala Leu Leu Val
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Pro Thr Leu Phe Ser Phe Phe Ala Lys Ser Thr Leu Ile Asp Lys Tyr
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Lys Glu Val Gly Glu Ala Val Ala Lys Arg Phe His Leu Pro Gly Ile
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Arg Gln Gly Tyr Gly Leu Thr Glu Thr Thr Ser Ala Ile Leu Ile Thr
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Pro Glu Gly Asp Asp Lys Pro Gly Ala Val Gly Lys Val Val Pro Phe
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Phe Glu Ala Lys Val Val Asp Leu Asp Thr Gly Lys Thr Leu Gly Val
370   375   380

Asn Gln Arg Gly Glu Leu Cys Val Arg Gly Pro Met Ile Met Ser Gly
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Tyr Val Asn Asn Pro Glu Ala Thr Asn Ala Leu Ile Asp Lys Asp Gly
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Trp Leu His Ser Gly Asp Ile Ala Tyr Trp Asp Glu Asp Glu His Phe
                      420   425   430

Phe Ile Val Asp Arg Leu Lys Ser Leu Ile Lys Tyr Lys Gly Tyr Gln
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Val Ala Pro Ala Glu Leu Glu Ser Ile Leu Leu Gln His Pro Asn Ile
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Phe Asp Ala Gly Val Ala Gly Leu Pro Asp Asp Asp Ala Gly Glu Leu
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Pro Ala Ala Val Val Val Leu Glu His Gly Lys Thr Met Thr Glu Lys
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Glu Ile Val Asp Tyr Val Ala Ser Gln Val Thr Thr Ala Lys Lys Leu
                      500   505   510

Arg Gly Gly Val Val Phe Val Asp Glu Val Pro Lys Gly Leu Thr Gly
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       plasmid backbone, with AAV2 internal terminal repeats that flank
       expression cassette; referred to as AAV2/5 U6 lucRI-1a

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       expression cassette; referred to as AAV2/5 U6 lucRI-1b

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caaccctatc tcggtctatt cttttgattt ataagggatt tttccgattt cggcctattg    1920

gttaaaaaat gagctgattt aacaaaaatt taacgcgaat tttaacaaaa tattaacgtt    1980

tataatttca ggtggcatct ttcggggaaa tgtgcgcgga acccctattt gtttattttt    2040

ctaaatacat tcaaatatgt atccgctcat gagacaataa ccctgataaa tgcttcaata    2100

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cgagcgtgac accacgatgc ctgtagtaat ggtaacaacg ttgcgcaaac tattaactgg    2700

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agaccccgta gaaaagatca aaggatcttc ttgagatcct tttttctgc gcgtaatctg        3180

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<210> 7
<211> 3923
<212> DNA
<213> Artificial

<220>
<223>  sequence for recombinant adeno-associated viral vector, including
       plasmid backbone, with AAV2 internal terminal repeats that flank
       expression cassette; referred to as AAV2/5 U6/U6 lucRIU6-3

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ttgtgagcgg ataacaattt cacacaggaa acagctatga ccatgattac gccagattta       240

attaaggctg cgcgctcgct cgctcactga ggccgcccgg gcaaagcccg ggcgtcgggc        300

gacctttggt cgcccggcct cagtgagcga gcgagcgcgc agagagggag tggccaactc        360

catcactagg ggttccttgt agttaatgat taacccgcca tgctacttat ctacgtagcc        420

atgctctagg aagatcggaa ttcgccctta agctagcccc cagtggaaag acgcgcaggc        480

aaaacgcacc acgtgacgga gcgtgaccgc gcgccgagcc caaggtcggg caggaagagg        540

gcctatttcc catgattcct tcatatttgc atatacgata caaggctgtt agagagataa       600
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ttagaattaa tttgactgta aacacaaaga tattagtaca aaatacgtga cgtagaaagt    660

aataatttct tgggtagttt gcagttttaa aattatgttt taaaatggac tatcatatgc    720

ttaccgtaac ttgaaagtat ttcgatttct tggctttata tatcttgtgg aaaggacgaa    780

acaccttttt cttacgctga gtacttcgat ttttggtgtt tcgtcctttc cacaagatat    840

ataaagccaa gaaatcgaaa tactttcaag ttacggtaag catatgatag tccattttaa    900

aacataattt taaaactgca aactacccaa gaaattatta ctttctacgt cacgtatttt    960

gtactaatat ctttgtgttt acagtcaaat taattctaat tatctctcta acagccttgt    1020

atcgtatatg caaatatgaa ggaatcatgg gaaataggcc ctcttcctgc ccgaccttgg    1080

gctcggcgcg cggtcacgct ccgtcacgtg gtgcgttttg cctgcgcgtc tttccactgg    1140

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gtagcatggc gggttaatca ttaactacaa ggaacccta gtgatggagt tggccactcc     1260

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actcaaccct atctcggtct attcttttga tttataaggg attttccga tttcggccta     1920

ttggttaaaa aatgagctga tttaacaaaa atttaacgcg aattttaaca aaatattaac    1980

gtttataatt tcaggtggca tctttcgggg aaatgtgcgc ggaacccta tttgtttatt     2040

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acactattct cagaatgact tggttgagta ctcaccagtc acagaaaagc atcttacgga    2460
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tggcatgaca gtaagagaat tatgcagtgc tgccataacc atgagtgata acactgcggc      2520

caacttactt ctgacaacga tcggaggacc gaaggagcta accgcttttt tgcacaacat      2580

gggggatcat gtaactcgcc ttgatcgttg gaaccggag ctgaatgaag ccataccaaa       2640

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tggcgaacta cttactctag cttcccggca acaattaata gactggatgg aggcggataa      2760

agttgcagga ccacttctgc gctcggccct tccggctggc tggtttattg ctgataaatc      2820

tggagccggt gagcgtgggt ctcgcggtat cattgcagca ctggggccag atggtaagcc      2880

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acagatcgct gagataggtg cctcactgat taagcattgg taactgtcag accaagttta      3000

ctcatatata ctttagattg atttaaaact tcatttttaa tttaaaagga tctaggtgaa      3060

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ccttctagtg tagccgtagt taggccacca cttcaagaac tctgtagcac cgcctacata      3360

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ttatagtcct gtcgggtttc gccacctctg acttgagcgt cgatttttgt gatgctcgtc      3720

agggggggcgg agcctatgga aaaacgccag caacgcggcc tttttacggt tcctggcctt     3780

ttgctgcggt tttgctcaca tgttctttcc tgcgttatcc cctgattctg tggataaccg      3840

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<210>  8
<211>  3589
<212>  DNA
<213>  Artificial

<220>
<223>  sequence for recombinant adeno-associated viral vector, including
       plasmid backbone, with AAV2 internal terminal repeats that flank
       expression cassette; referred to as AAV2/5 U6 lucRI-4(sense)

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atgctctagg aagatcggaa ttcgccctta agctagcccc cagtggaaag acgcgcaggc    480

aaaacgcacc acgtgacgga gcgtgaccgc gcgccgagcc caaggtcggg caggaagagg    540

gcctatttcc catgattcct tcatatttgc atatacgata caaggctgtt agagagataa    600

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aataatttct tgggtagttt gcagttttaa aattatgttt taaaatggac tatcatatgc    720

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cgaccaaagg tcgcccgacg cccgggcttt gcccgggcgg cctcagtgag cgagcgagcg   1020

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ttatcccctg attctgtgga taaccgtatt accgcctttg agtgagctga taccgctcgc      3540

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<210>  9
<211>  3589
<212>  DNA
<213>  Artificial

<220>
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<223> sequence for recombinant adeno-associated viral vector, including
      plasmid backbone, with AAV2 internal terminal repeats that flank
      expression cassette; referred to as AAV2/5 U6 lucRI-4(antisense)

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       plasmid backbone, with AAV2 internal terminal repeats that flank
       expression cassette; referred to as AAV2/2 U6 eGFPRI-1a

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<213> Artificial

<220>
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       plasmid backbone, with AAV2 internal terminal repeats that flank
       expression cassette; referred to as AAV2/5 poll lucRI

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       expression cassette; referred to as AAV2/5 U6/U6 lucRI-2

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